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EFFECTS OF METHAMPHETAMINE ON LOCOMOTOR ACTIVITY AND THALAMIC GENE EXPRESSION IN LEPTIN-DEFICIENT OBESE MICE

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Abstract

Leptin is an adipose-derived hormone that regulates energy balance. Leptin receptors are expressed in extrahypothalamic sites and several reports showed that leptin can influence feeding and locomotor behavior via direct actions on dopaminergic neurons. The leptin deficient mouse (*ob/ob*) has been used as an animal model of blunted leptin action, and presents with obesity and mild type 2 diabetes. We used *ob/ob* mice to study the effect of repeated 7-day methamphetamine (METH) administration analyzing locomotion, behavioral sensitization, and somatosensory thalamic mRNA expression of voltage-gated calcium channels and glutamatergic receptors using RT-PCR. We observed reduced METH-mediated responses in *ob/ob* mice associated with enhanced mRNA expression of key voltage-gated and glutamate receptors in the somatosensory thalamus. Results described here are important for understanding the control of locomotion and thalamocortical excitability by leptin.

Keywords

Methamphetamine; thalamic nuclei; *ob/ob* mouse

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Conflict of interest

Authors report no financial conflict of interest, or otherwise, related directly or indirectly to this study.

Introduction

Leptin is an adipose-derived hormone [1] known to control appetite and energy expenditure [2]. Leptin-deficient mice presenting a mutation in the ‘obese’ gene (i.e., homozygous *ob/ob* mice) were described to develop severe obesity after the fifth postnatal week and to exhibit decreased energy expenditure and hyperphagia [2,3]. Leptin receptors are predominantly expressed in the hypothalamus, although they have also been described to be expressed in extrahypothalamic areas like somatosensory thalamic nuclei and the mesolimbic dopamine system [4–6]. It has been found that in *ob/ob* mice dopamine is required for hyperphagia [7,8] while manifesting hypoactivation of the dopaminergic system [9] that includes disruption in striatal dopamine D2 receptors (D2R) [10].

Methamphetamine (METH) abuse disrupts dopamine neurotransmission and induces neurophysiological and cognitive alterations in humans [11,12]. Our group has described multiple METH-mediated alterations in mouse cortical and sub-cortical areas [13–16], including changes in mRNA levels of membrane receptors and voltage-gated ion channels in medial prefrontal neurons [17]. Repeated exposure to cocaine, a psychostimulant that can also increase DA neurotransmission, enhanced low threshold T-type calcium channel protein levels in mouse somatosensory thalamus neurons [18]. Selective targeting of T-type calcium channels using specific blockers reduced cocaine-mediated hyperlocomotion concomitantly with a reduction in GABAergic neurotransmission onto thalamic ventrobasal nucleus [19], thus suggesting the involvement of somatosensory ventrobasal thalamic nucleus in locomotor alterations.

We hypothesized that leptin acts as a neuromodulator of psychostimulant-mediated changes in locomotor activity and thalamocortical excitability mediated by the alteration changes in mRNA expression of key voltage-gated calcium channels (P/Q-type and T-type), hyperpolarization-activated cyclic nucleotide-gated channels (HCN2), and glutamate receptor subunits (AMPA and NMDA) [16–19]. Behavioural analyses described that both genotypes (wildtype and *ob/ob*) showed METH-induced locomotor sensitization (increased locomotion after 7-days compared to day 1) but with lower raw values for *ob/ob*. In addition, METH-treated *ob/ob* mouse thalamic tissue showed altered mRNA expression of voltage-gated calcium channels, hyperpolarization-activated cyclic nucleotide-gated channels, and glutamate receptor subunits compared to wildtype thalamic neurons. Our results suggest that leptin deficiency can alter transcriptional regulation in the thalamus and influence basal and METH-elicited locomotor behavior.

Materials and Methods

Animals

We used female C57BL/6JF_{cn} wild type (WT) mice (3–6 months old, Central Animal Facility at University of Buenos Aires, animal protocol #50–2015) or leptin-deficient, homozygous B6.Cg-Lep^{ob/J}, obese *ob/ob* mice (kindly provided by Dr. J.J. Poderoso, (INIGEM, CONICET-UBA)). Females were housed four per cage in the absence of males, a condition known to maintain them acyclic. Genotyping of *ob/ob* littermates was determined during the second postnatal week according to Finocchietto *et al.* [20]. Principles of animal

care were in accordance with the ARRIVE guidelines and CONICET (2003), and approved by its authorities using OLAW/ARENA directives (NIH, Bethesda, MD, USA).

Drug treatments

(+)-Methamphetamine hydrochloride (Sigma, St Louis, MO) was administered subcutaneously (sc) once a day for 7 days (1 mg/Kg, calculated as free base, dissolved in sterile saline solution). The METH regimen used in this study was performed according to studies by González et al. [15]. Vehicle groups received the same volume of sterile saline.

Behavioral studies

Mouse locomotor activity was recorded with an automated system (Ethovision XT 7.0, Noldus, The Netherlands) as previously described [15,18,19]. Total distance traveled (cm) was quantified for a total of 5 minutes prior to injections (basal), and 40 minutes following the last injection of METH/vehicle for days 1 and 7 (test). Drug-induced locomotion was expressed as the ratio between locomotion following drug injection and basal locomotion. Locomotor sensitization was calculated as the ratio of relative locomotion after the 7-day treatment compared to day 1.

Real time PCR

Mice were killed 4 days after treatments and their brains were rapidly removed. The somatosensory thalamic complex (ventrobasal+reticular nuclei) was dissected, placed on dry ice, and stored at -70°C in RNAlater for further assays. Total RNA was isolated using TRIZOL reagent (Invitrogen) according to the manufacturer's protocol. Five hundred nanograms of RNA were treated with DNaseI (Deoxiribonuclease I) and reverse-transcribed in a 20 μL reaction using M-MLV reverse transcriptase and random hexamers. For quantitative real-time PCR (qPCR) primer sets were designed for the specific amplification of murine *Cacna1a*, *Cacna1g*, *Cacna1i*, *Hcn2*, *Gria1*, *Grin1*, and *Gapdh* as a housekeeping control gene [17]. Each sample was assayed in duplicate using 4 pmol of each primer, 1 \times SYBR Green Master Mix, and 2–20 ng of cDNA in a total volume of 13 μL . Amplification was carried out in an ABI PRISM 7500 Sequence Detection System.

Statistical analysis

InfoStat software (www.infostat.com.ar) was used for statistical comparisons. Statistics were performed using two-way (genotype and treatment) ANOVA followed by Bonferroni post hoc tests. Differences were considered significant if $p < 0.05$.

Pharmacological reagents

Drugs were purchased from either Sigma (St. Louis, MO; USA) or Tocris (Ellisville, MO; USA). Reagents for RT-PCR were purchased from Qiagen (GermanTown, MD, USA), Applied Biosystems (Foster City, CA, USA) or ThermoFisher-Invitrogen (Waltham, MA, USA).

Results

We measured body weight of WT and *ob/ob* female mice at the beginning (day 1) and end (day 7) of treatment. Table 1 shows that at both day 1 and day 7 *ob/ob* mice (OB) were significantly heavier than WT littermates. Paired t-test within each group comparing day 1 vs. day 7 showed no treatment effect on body weight.

We started assessing basal locomotor activation of either WT and *ob/ob* transgenic mice before METH administration at day 1. Obese *ob/ob* female mice showed substantially diminished locomotion throughout the arena compared to wild type mice (Figure 1B, left). After the first METH administration on day 1, both WT and *ob/ob* mice increased their locomotion (Figure 1B, right), while maintaining differences between genotypes. At day 7, basal locomotor activity showed interactive effects between genotype and treatment, and we found differences between WT-vehicle and OB, but no changes in locomotion after METH between genotypes (Figure 1C, left). METH-mediated stimulation of locomotor activity was significantly higher at day 7 (Figure 1C, right). METH-mediated sensitization (i.e., increased METH-induced motor activation at day 7 administration compared to day 1) was observed in both WT and *ob/ob* mice (Figure 1D).

We evaluated mRNA expression of voltage-gated calcium channels, hyperpolarization-activated cyclic nucleotide-gated (HCN2) channels, and glutamate receptor subunits in the somatosensory thalamic complex (ventrobasal+reticular nuclei) four days after METH treatment (Figure 2). Assays of P/Q-type $Ca_v2.1$ (*Cacna1a*), and T-type $Ca_v3.3$ (*Cacna1i*) voltage-gated calcium channel subunits showed higher mRNA levels in *ob/ob* mice relative to WT (Figure 2A,C). No changes were detected for assays of T-type $Ca_v3.1$ mRNA levels (*Cacna1g*) (Figure 2B). The assays of HCN2 showed elevated mRNA levels in *ob/ob* that were down-regulated by METH (Figure 2D). Assays of NMDA-GluN1 *Grin1* (Figure 2E) and AMPA-GluA1 *Gria1* (Figure 2F) mRNA levels were observed to be higher in *ob/ob* mice, independently of METH administration.

Discussion

Results presented here support the hypothesis that the absence of leptin regulation during development alters psychostimulant-mediated locomotor activity and thalamocortical excitability. In the present study we show that repetitive administration of METH induced lower locomotor stimulation in leptin-null, *ob/ob* mice. This effect was also observed in the absence of METH. It should be noted that locomotor activity tends to be depressed in obese mice, probably due to abnormal neuroendocrine function [21]. Locomotor responses to systemic administration of 10 mg/kg cocaine was reduced in *ob/ob* mice, while conditioned place preference was increased by 2.5 mg/kg of cocaine [22]. These results contributed new important information about METH effects on females, extending our group's previous report [14].

Dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc), and its control by the pedunclopontine nucleus [4], mediate psychostimulant stimulated locomotor activity [23]. Dopaminergic modulation is also required for feeding in

ob/ob mice [7–9]. Exogenous leptin administration has been shown to reduce repetitive action potential discharge in VTA [24] and pedunculopontine neurons [25,26]. Therefore, in leptin-deficient *ob/ob* mice there may be inhibitory mechanisms that compensate for leptin-mediated inhibition. D2 receptors are required for locomotor sensitization [27]. Reduced striatal D2R binding described for *ob/ob* mice [10] might explain the lower locomotor activity reported here after 7 day METH administration. In conclusion, it may be suggested that lack of circulating leptin during development blunted the *ob/ob* response to METH administration.

Voltage-gated T-type calcium channels expressed in the somatosensory thalamocortical system have been involved in psychostimulant-induced locomotor activation [19,28]. Here, we have evaluated mRNA expression of a wide range of voltage-gated calcium channels (T and P/Q types), HCN2, and glutamate receptor subunits in the somatosensory thalamic complex after METH treatment in both WT and *ob/ob* mice. Higher mRNA expression levels for voltage-gated Ca_v2.1 and Ca_v3.3 calcium channels and NMDA-GluN1, and AMPA-GluA1 glutamate receptor subunits were observed in somatosensory thalamic neurons in *ob/ob* mice. METH-administration did not influence those changes. No significant changes were observed for Ca_v3.1 calcium channels. Increased mRNA levels were also observed for HCN2 channels in *ob/ob* mice. Interestingly, METH administration reduced HCN2 levels in the sensory thalamus of *ob/ob* mice. Further experiments are still needed in order to quantify protein levels changes of these channels/receptors mediated by METH.

These transcriptional alterations observed in thalamic nuclei from *ob/ob* mice might underlie previously described impaired sleep consolidation [29]. Indeed, Ca_v2.1 voltage-gated calcium channels are located in the dendrites of ventrobasal neurons and support *gamma band* oscillations that modulate membrane depolarization towards action potential threshold [30]. Thalamic reticular neurons express Ca_v3.2 and Ca_v3.3 T-type calcium channel subunits [31]. Ca_v3.1 subunits containing T-type calcium channels are expressed at both pre-synaptic thalamic reticular axon terminals and post-synaptic ventrobasal cell bodies [32], and are implicated in the transitions between waking and slow sleep [33]. Moreover, HCN2 channels are involved in controlling thalamocortical activity, reducing the susceptibility to convulsant agents [34].

In addition, it was reported that somatosensory loss induces profound changes in motor adaptation and anticipation in humans [35]. Therefore, changes reported here on thalamic nuclei gene expression might underlie alterations in somatosensory processing that might in turn induce detrimental effects on locomotion. Further studies are needed on psychostimulant-mediated actions in this and other leptin resistance animal models to elucidate their effects on sensory and motivational brain networks.

Functional implications for thalamocortical rhythmicity

Voltage-gated ionic channels are responsible for neuronal excitability, endowing them with autorhythmic membrane oscillatory capabilities [36]. Synaptic receptors mediate functional contacts between neurons, spreading oscillations throughout neuronal networks. In such networks, autorhythmic neurons may either act as oscillators/pacemakers or as resonators

[36,37]. Changes described here for mRNA levels of voltage-gated ionic channels and synaptic receptors would affect neuronal oscillations in the CNS. Indeed, blocking or transgenic elimination of P/Q-type calcium channels have been described to prevent the generation of gamma band oscillations [30,37,38]. T-type and HCN channels are physically associated [39] to underlie low-frequency oscillatory activity at the thalamocortical level [33,38,39,40]. Indeed, T-type channels are key elements mediating the sensitivity to psychostimulants [19,28], consistent with the observed enhancement in low frequency oscillatory activity after METH [41,42] and changes in protein levels of T-type channels mediated by cocaine [43] administration. Further studies are needed to clarify METH-dependent alterations of voltage-gated ionic channels and glutamate receptors protein levels.

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Abbreviations

METH	methamphetamine
DA	dopamine
OB	obese <i>ob/ob</i> mouse
TRN	thalamic reticular nucleus
VB	ventrobasal.

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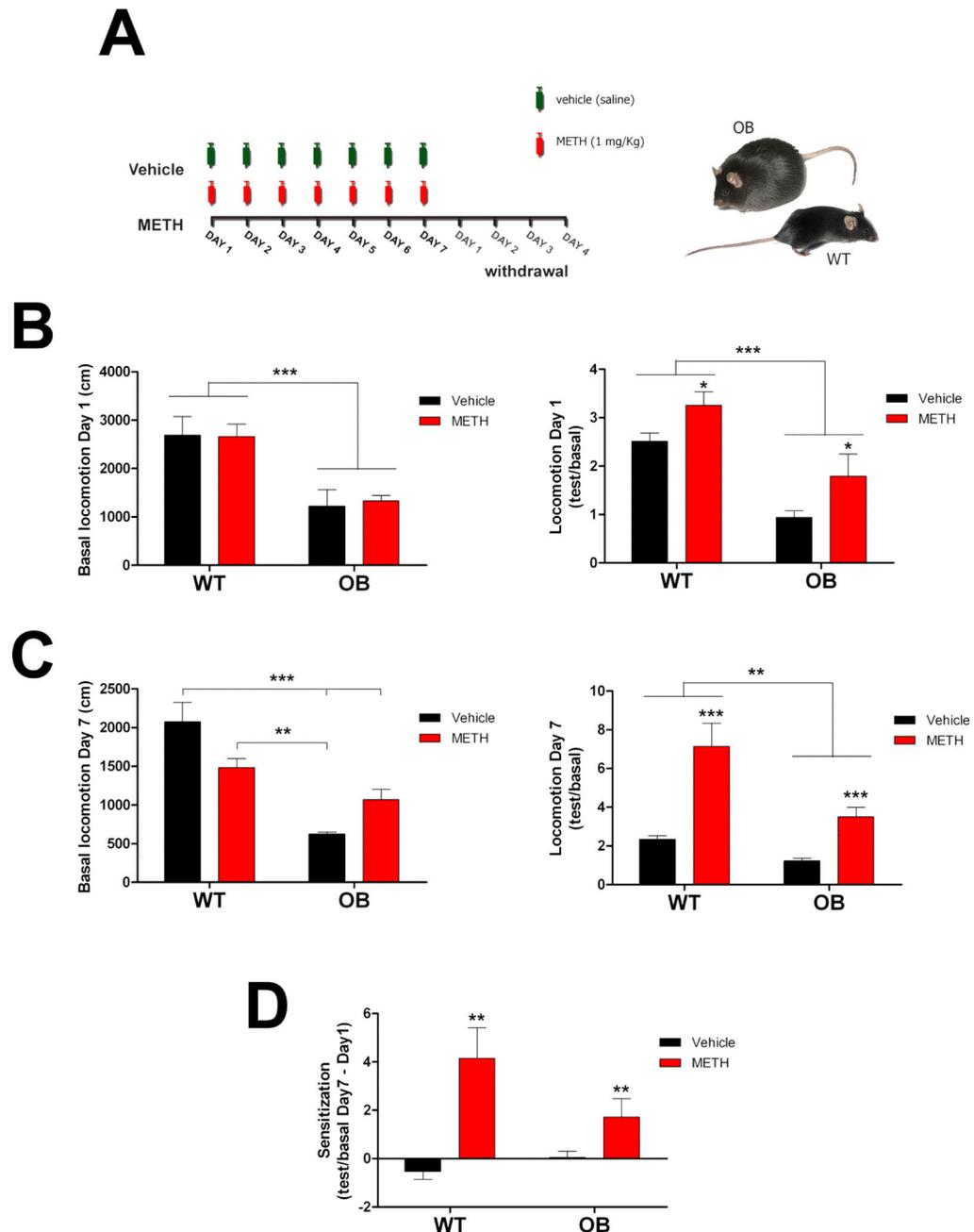


Figure 1.

A) Schematic figure showing the METH administration protocol used.

Methamphetamine (METH) or vehicle were administered once a day for 7 consecutive days.

The ventrobasal thalami were collected 4 days after the last injection. **B) Locomotor response at Day 1.** Basal (left panel; two-way ANOVA, $F_{(3,21)}=8.34$ $p<0.001$; genotype effect $F_{(1,21)}=24.97$ $p<0.0001$ comparing WT vs. OB) and drug-induced (right panel; two-way ANOVA, $F_{(3,21)}=7.93$ $p<0.01$; genotype effect $F_{(1,21)}=19.08$ $p<0.001$, WT vs. OB; treatment effect $F_{(1,21)}=5.27$ $p<0.05$, vehicle vs. METH administration) relative locomotion (test/basal) on day 1. **C) Locomotor response at Day 7.** Basal (left panel; two-way

ANOVA, (N=5–6), $F_{(3,21)}=16.16$ $p<0.0001$; interaction effect $F_{(1,21)}=11.95$, $p<0.01$, Bonferroni post-test among all groups) and drug-induced (right panel; two-way ANOVA, (N=5–6), $F_{(3,21)}=13.09$ $p<0.0001$; genotype effect $F_{(1,21)}=10.86$ $p<0.01$, WT vs. OB; treatment effect $F_{(1,21)}=24.14$, $p<0.001$, vehicle vs. METH administration) relative locomotion (test/basal) on day 7.. **D) Locomotor sensitization.** Relative locomotion day 7 – day1. Two-way ANOVA, (N=5–6), $F_{(3,21)}=13.09$ $p<0.0001$; treatment effect $F_{(1,21)}=14.21$, $p<0.01$, vehicle vs. METH administration).

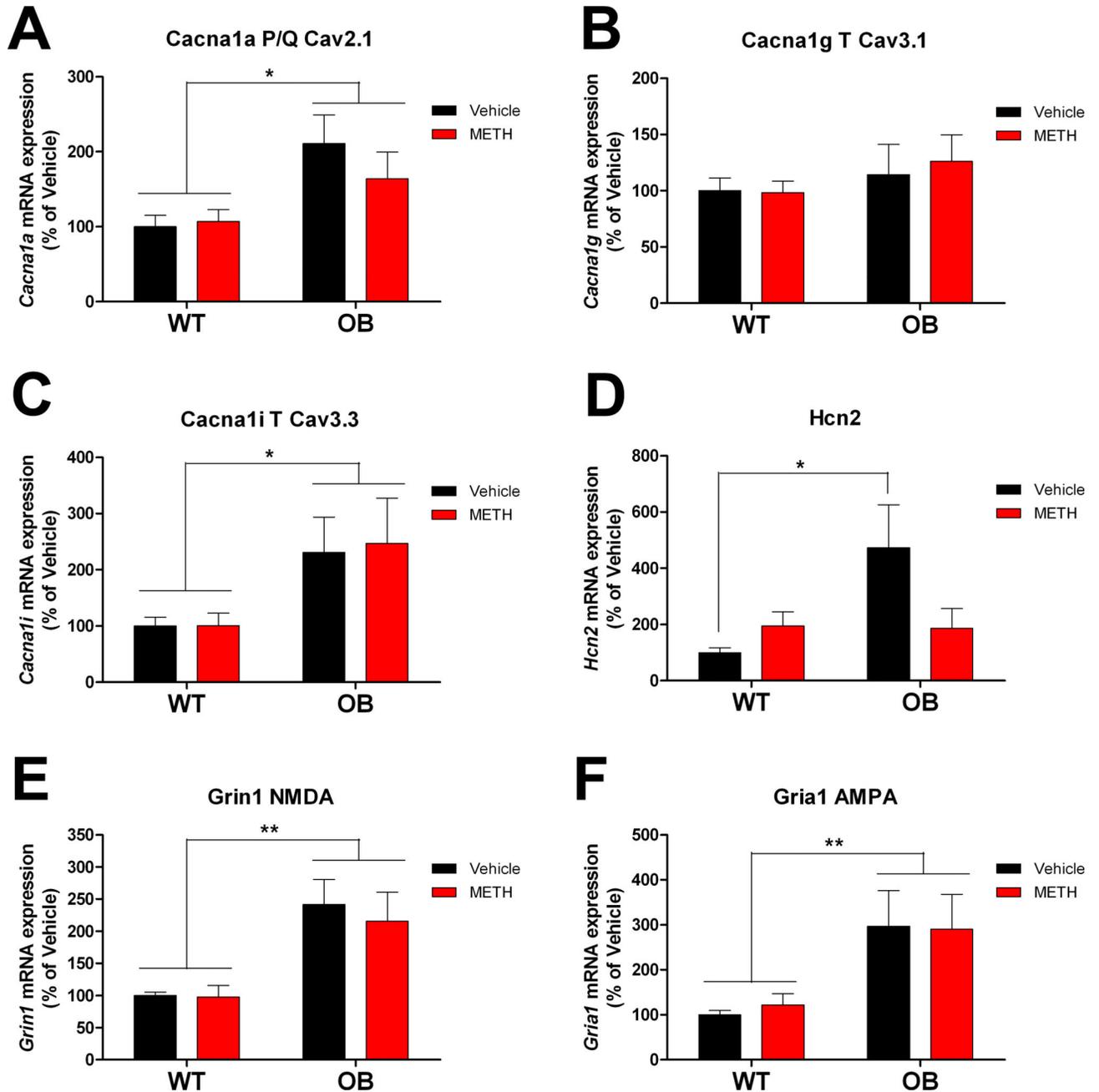


Figure 2. mRNA level expression in ventrobasal thalamic nucleus evaluated by RT-PCR
A–C, mRNA level expression of voltage-gated calcium channels subunits *Cacna1a* (A; P/Q Cav2.1), *Cacna1g* (B; T Cav3.1), *Cacna1i* (C; T Cav3.3) (Two-way ANOVA, (N=5), *Cacna1a* $F_{(3,19)}=3.98$, $p<0.05$; genotype effect $F_{(1,19)}=6.86$, $p<0.05$ WT vs OB; *Cacna1i* $F_{(3,19)}=3.09$, $p<0.05$; genotype effect $F_{(1,19)}=8.33$, $p<0.05$ WT vs OB). **D**, mRNA level expression of HCN subunits *Hcn2* (Two-way ANOVA, $F_{(3,19)}=5.00$, $p<0.05$; interaction effect $F_{(1,19)}=8.22$, $p<0.05$). **E**, mRNA level expression of glutamate receptors subunits *Gria1* and *Grin1* (AMPA GluA1 and NMDA GluN1, respectively; two-way ANOVA, *Gria1*

$F_{(3,19)}=6.06$, $p<0.01$; genotype effect $F_{(1,19)}=16.44$, $p<0.01$ WT vs OB; *Grin1* $F_{(3,19)}=5.29$, $p<0.05$; genotype effect $F_{(1,19)}=15.13$, $p<0.01$ WT vs OB;).

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Table 1

Body weight at the beginning (day 1) and end (day 7) of treatment.

	Body weight (gr)			
	WT vehicle	WT METH	OB vehicle	OB METH
Day 1	26.7 ± 1.0	25.7 ± 1.1	60.1 ± 3.0	64.8 ± 1.6
Day 7	26.4 ± 1.0	25.0 ± 1.0	59.2 ± 2.1	63.8 ± 1.4

Two-way ANOVA-Bonferroni (N=5–6); day 1: $F(3,21)=141.94$ $p<0.0001$; genotype effect $F(1,21)=412.87$ $p<0.0001$ comparing WT vs. OB; day 7: $F(3,21)=156.33$ $p<0.0001$; genotype effect $F(1,21)=454.02$ $p<0.0001$ comparing WT vs. OB)